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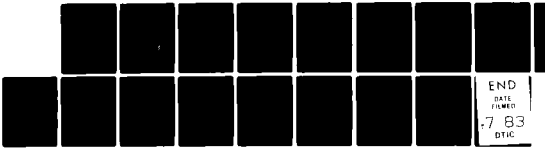

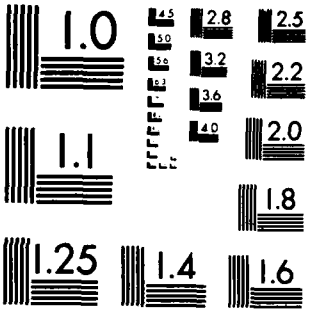

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EVOKED POTENTIALS TO EVALUATE MECHNISMS OF PERIPHERAL NERVE REPAIR

Final Report

David G. Kline, M.D.

February 1980

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Report covers a 10 year period of research both laboratory and clinical concerned with peripheral nerve injuries, their evaluation electrically and their management operatively. Studies delineating the value of each evoked nerve action potential recording for the most frequent injury type, - the lesion incontinuity are summarized as are studies on the effect of partial or fractional injury to nerve, removal of collateral

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blood supply or mobilization of the injured nerve, and various types of repair to nerve along with preliminary studies on the timing of repair. Stressed is the fact that many further important laboratory and clinical studies are needed and have been stimulated by some of these investigations.

Final Report for Contract DADA 17-69-C-9133 and DMD 17-76-C-6035
Evoked Potentials to Evaluate Mechanisms of Nerve Repair.

Summary of previous PERIPHERAL NERVE RESEARCH Involving the Investigators as backround.

Since peripheral nerve regeneration and peripheral nerve repair research has over the years utilized all sorts of species of animals, work between 1962 and 1964 focused on the response of various species to different nerve injuries. This work was carried out at the Walter Reed Army Institude of Research in Washington, D.C. Response of the primate in terms of degree of connective tissue proliferation, axonal disorganization, and maturation was closer to that of the human (The Lyons-Woodall Collection of Woeld War II was utilized at the Armed Forces Institute of Pathology) than that of the dog or lower phylum primate. Towards the end of this period, Primates were used to study a potentially resorable wrapper or cuff for nerve repair and subsequent studies in that laboratory helped develop silastic for this purpose. During this period it became apparent to the principal investigator, as it had previously to others, that most injuries, either military or civilian, do not completely transect nerve nor destroy its external continuity but do destroy or change to a variable extent the internal architecture of the nerve. Objective evaluation of these clinically frequent lesions in continuity had to await regeneration of axons not only through the injury but down the distal stump and into muscular or sensory inputs. Thus, whether evaluated clinically or electrically many months had to pass before useful evidence of regeneration could be obtained. Histologic studies indicate that there was a significant number of axons, often of surprisingly large caliber, in the distal stump particularly just beyond the injury. These axons could be seen in the early weeks to several months after injury or repair even though a significant number of sizable ones were absent in the more distal stump or in the end-input regions. These observations suggested the possibility of stimulating nerve either proximal or immediately distal to the injury and recording on the other side of the injury to see if a conducted response could be evoked through nerve undergoing early regeneration. In vitro nerve action potential(NAP) recording had, of course, been reported and dates back to Erlanger and Gasser who described the use of the oscilliscope for such studies. However, if NAP recording was to be of clinical use, it needed to be adapted to in vivo use. An evoked nerve action potential technique used in vivo on injured but regenerating primate nerve was studied at the University of Michigan between 1965 and 1967. Bipolar stimulating and recording electrodes were used and were held by clamps. Electrode tips were placed around the injured but regenerating nerve which was suspended on them without immersing the nerve in oil or killing one end of the nerve. It was found that such recordings could be made on intact nerves and that muscle action potential artifact could be separated from the nerve action potential by proper oscilloscope sweep speeds. Stimulus used was found best at the microsecond level so as to minimize stimulus artifact since stimulation and recording distances were brief compared to those used for more physiologic studies. Stimulus was delivered and recorded by bipolar electrodes and picked up by differential amplifiers, displayed on an oscilloscope, and photographed by a Polariod camera. Crush, as well as sever and suture injuries, were studied in the primate (Rhesus monkey). By six weeks post-crush, nerve action potentials could be recorded up to six centimeters distal to the injury even though planter flexion was absent on clinical testing and on stimulation of the nerve. In sutured nerves, nerve action potentials could be recorded just beyond the injury by eight to ten weeks even though planter flexion did not come back for several more months. The nerve action potentials in this study were correlated with the axon count of the distal stump recording sites, as well as with those of the proximal

** Abstracted from a submission to NINCDS for a Nerve Center grant to replace funding lost on these on-going projects.*

stimulating sites. Care was taken to evaluate the axon population, not only as to number, but as to size and axons were placed into three categories: small- less than six microns in diameter, moderate- six to ten microns, large- greater than ten in diameter.

Since bipolar recordings were utilized, amplitude and form of the NAP could only be correlated in a semi-quantitive fashion with axon counts and axon distribution. Impression from these as well as subsequent studies was that several thousand moderate sized (6-10 microns in diameter) fibers were necessary to be able to evoke and record a nerve action potential using a non-computerized, non-averaging system.

Studies begun at LSU in 1967, and again in primates, demonstrated that distal stump nerve action potentials could be recorded from re generating nerves weeks before there was any electromyographic evidence of reinnervation. Thus, a distal stumo nerve action potential could be seen even though reversal of deinnervational changes in muscle or presence of an evoked muscle action potential were absent. Thus, and this was borne out by companion histologic studies as well, axons could be present in large numbers and be relatively mature within the distal stump at a time when muscle had not been sufficiently reinnervated to provide electromyographic let alone clinical evidence of recovery. *Support for this and subsequent work to be described was gained from the U. S. Department of Defense and the U. S. Army Research and Development Command in 1969 and extended to November of 1977.*

WORK COMPLETED WHILE FUNDED BY U.S. ARMY

Clinical work evaluating intraoperative electrical study of lesions in continuity had begun in 1966 in Ann Arbor and has been continued here at LSU in New Orleans under DADA 17-69-C-9133. It has been recognized for many years that some lesions in continuity regenerate adequately enough to recover without resection and suture while others do not and the clinical challenge has been to identify which is which since appearances can be deceiving. Since there are no reliable clinical clues in patients with a severe or complete deficit distal to the lesion, recording across the lesion intraoperatively has been utilized. Of course, a large bulbous appearing neuroma will be more likely to have disorganized axons which have branched and which are therefore regenerating poorly than one that is not quite so large, but "looks can be deceiving" and a bulbous appearance and feel to palpation may actually be caused by a proliferation of epineurial and/or perineurial connective tissue while intrafasicular anatomy may be relatively organized and composed of relatively mature axons with minimal branching or straying. On the other hand, neuromas which are small and thus appear innocuous to both inspection and palpation may have enough endoneurial scarring and intrafasicular neurotmsis to preclude successful regeneration. If, in these situations one takes a "wait and see" attitude and looks for evidence of satisfactory recovery by stimulating the nerve without recording, by electromyographic recovery, or by repetitive clinical examination many months may pass and if good recovery does not ensue then it is often too late for surgical repair with satisfactory results because the distla motor innervation sites will have undergone irreversible atrophy.

Operative studies of nerve action potential recordings, stimulation observations, and neuropathology have been and are being correlated with pre- and postoperative electrical and clinical studies. Since operative recording was done for other surgeons as well as for our own use, and since information acquired at the operating table was not always followed, data on a few nerves resected despite evidence of NAP activity is available and indicates that such nerves would have done

well if only a neurolysis and not a resection and suture was done. Conversely, clinical data is also available in a small group of nerves left without resection despite, after an appropriate interval from injury, absence of nerve action potentials, and these with one exception have not made a satisfactory clinical, functional recovery. Conversely, specimens correctly resected because of little or no evoked NAP activity across the lesion shows a pattern of axonal disorganization and immaturity as well as sufficient enough interfasicular scarring to permit one to predict a poor functional result.

Clinical experience over a nine-year period with evoked nerve action potentials as well as other electrical intraoperative and non-operative electrical studies as well as experience with transecting injuries was drawn together in the early fall of 1976.

In order to provide an adequate followup period, the series of patients selected for this summary extended from June, 1966 until June, 1975, and thus there was at least one year followup on each patient. Sixty-five percent of the patients had three or more years of followup, while eighty percent had two or more years, and the remainder had at least one year. The series included 270 patients with 315 injured or lesion-involved elements. Excluded were cranial nerve injuries, superficial or sensory nerve injuries, and nerve injuries involving calf or ankle or foot. In addition to reviewing pertinent neurophysiologic information available concerning the behavior of injured and regenerating nerve and the proper selection of electrodiagnostic studies, conclusions in this article include: 1) indications for primary repair of lacerated nerves particularly those lacerations involving proximal brachial plexus and sciatic complex, 2) one-quarter to one-third of lacerating injuries still leave the nerve with some degree of gross continuity, 3) need for relatively acute management of aneurysms and fistulae associated with nerve injury, tight space syndromes due to fracture and contusion, foreign bodies lodged in or near nerve, blood clots under pressure, and selected pain problems and tumors was stressed, 4) personal experience with 255 injured neural elements in continuity due to gunshot wound, contusion, and injury associated with fractures and stretch was reviewed. Importance of electrophysiologic testing as well as clinical evaluation was stressed since serial electrical testing placed 65 injured neural elements into a different treatment category than would have been predicted from clinical examination alone. For example, as can be seen in Table #I, in a group of 155 lesions in continuity felt to be complete on a clinical basis, intraoperative studies such as stimulation and nerve action potential recording showed that 55 were regenerating. These lesions fared well as a result of neurolysis only rather than resection and suture. These patients, of course, gained a functional result superior to what would have been predicated had resection and suture been done. As can be seen in Table #II, in a category of 100 lesions felt to be incomplete clinically, 10 had no response to stimulation nor a nerve action potential and resection and repair were necessary rather than neurolysis. The clinical appearance of a partial lesion was due to overlap or shared innervation from a nearby nerve. Histologically, the resected specimens in this group showed a degree of neurotmesis incompatible with successful functional regeneration without resection. For the last few years, we have operated on approximately 80 new patients per year with nerve lesions. These patients are worked up as thoroughly as possible with electrical as well as clinical studies. Information concerning each patient is placed in a computer system which now holds data on close to 500 patients. The data encompasses nerve or nerves injured, level of injury, injuring force, date of injury, results of preoperative electrical studies, results of intraoperative electrical studies, operation performed, histology of the lesion where available, and postoper-

ative results along with the period of followup that it took to gain it.

Partial Injury to Nerve

By 1969, it was apparent that the NAP and evoked EMG techniques evolved to evaluate lesions in continuity could also be used to study the time course of various experimental injuries. Prior experimental work studying regeneration after injury almost always focused on complete lesions such as total severance, severance and suture, or total cross-section crush. Since such complete lesions lead to complete Wallerian degeneration, prior studies focused on regeneration with this pattern as a backround. Therefore, it was flet important to preserve a measurable portion of the axonal volume so that an experimental model would be provided for not degeneration but also regeneration in a partially injured nerve.

After recording baseline evoked nerve and muscle action potentials, tibial nerves in both lower extremities of 12 primates were partially lacerated. Lacerations were monitored electrically during the injury so that NAP amplitude on one side was reduced to 75% of that recorded prior to injury while that on the other side was reduced to 50%. Similar recordings were repeated at one interval of 2, 6, 8, 12, or 16 weeks post partial laceration, and several nerves were studied hourly for an 8-hour period immediately after injury. In addition to the reduction of NAP amplitude seen immediatley after injury, there was further reduction of amplitude with time. Conduction velocity although not changed immediately after injury did decrease over the initial weeks after injury. In two nerves lacerated to 50% baseline function neither an evoked NAP nor EMG response could be recorded on followup study even though immediately after injury electrical responses had been present. In two other injuries evoked EMG response could not be recorded although an NAP could be. There was microscopic evidence of edema, demyelination, and degeneration of axons adjacent to the severed portion of the nerve. It was concluded that the effects of partial laceration on neural function are not limited to those seen immediately after injury but with time damage may extend and reduce function of axons originally left intact. Although conduction velocity did not change immediately after the lesion, it did when the nerve was restudied weeks later and decreases were more severe in the nerves with a 50% than with a 25 % laceration. This apparent extension of injury was somewhat puzzling for lacerations were made with a sharp knife and manipulation of the nerve was minimal. One might expect the fibers left behind to maintain their conduction with relatively fast latencies as they had in the early hours following injury. Histologic studies using the light microscope showed greater Wallerian changes at the distal stump recording sites than would have been predicted by the initial post injury evaluation at the same recording site and by the extent of the initial laceration. Instead, the distal stump histologic changes corresponded with NAP's recorded immediately prior to removal of the nerve for histologic study. The zone adjacent to the original site had axons which had undergone partial degeneration. Some axons had dropped out altogether while others appeared to be perfectly intact and well-preserved. It was concluded that the original injury extended to involve adjacent, partially intact axons.

Since edema, hemorrhage, or the presence of damaged tissue were possible causes for extension of the destruction produced by the original laceration, further fractional or partial injuries were carried out. Injury mechanisms selected included partial crush, injection of blood, and injection of saline into the nerve. Once again, neural function was monitored by evoked nerve action potentials and electromyographic tech-

niques prior, during, and after injury, and this data was correlated and compared with histologic data for each fractional injury. Saline injection initially reduced the amplitude of evoked NAP's but by two weeks post injection, amplitudes had returned to baseline and conduction velocity was maintained throughout the experiment. By comparison, nerves injected with blood had by two weeks, definite decreases in velocity in three of seven instances and an overall decrease in velocity which averaged 11.1%. Partially crushed nerves exhibited minimal extravasation of blood into the region of the injury but nonetheless had a marked decrease in both conduction velocity (33%) and NAP amplitude (16.5%) by two weeks post injury. It seemed from these studies that the presence of blood and/or damaged neural tissue might be responsible for extension of the injury.

Dexamethasone and Partial Injury

Since steroids, particularly high doses of a dexamethasone series steroid, are thought to reduce edema and/or prevent further edema elsewhere in the nervous system, a trial of this medication in partially injured primate nerves was undertaken. Fifty percent lacerations and forty percent crush injuries were created in tibial nerves of 20 Rhesus monkeys. Both tibial nerves in four animals were mobilized but not injured and served as controls. Immediately following injury, a short-term high dose regimen of dexamethasone was given to half the animals and saline to the others. The saline treated animals received 0.25 milliliters of normal sodium chloride IM every six hours for five days while steroid animals received 0.1 milligrams per kilogram of body weight of dexamethasone IM every six hours for five days.This dosage of dexamethasone would be equivalent to 28.0 milligrams per day in a 70 kilogram adult human. Specific treatment regimen for each animal was randomly selected by personnel other than the investigators and information concerning the treatment used was withheld until all electrophysiologic and histologic data had been analyzed. Records of NAP and EMG responses were made prior to injury, immediately after injury, at 15 minutes, and then 2 weeks after injury. Recordings were also made at 8 weeks in control nerves and in several partially crushed nerves. Extension of the partial laceration or crush occured whether the animal was treated with saline or with dexamethasone.

Blood supply and effect of mobilization on regenerating nerve

Since adequate repair of most nerve injuries requires that the surgeon make up length lost due to retraction of stumps and/or resection of a damaged length of tissue, evoked NAP and EMG techniques were used to study the effect of mobilization on the blood supply and regeneration of injured nerves. The literature had indicated that when carefully done, mobilization of intact nerves with killing of collateral circulation was innocuous, but was less clear on its effect on injured and/or regenerating nerve.

Both tibial nerves were partially crushed in six monkeys, completely crushed in 10, and severed and sutured in 12, and left intact in another 4 animals. Evoked nerve and muscle action potentials were monitored to document the extent of injury. The nerve on one side was then completely mobilized while the opposite nerve was not mobilized. At one interval of 1 to 52 weeks later, NAP and EMG studies were repeated and extremities were perfused with contrast gelatin and frozen for subsequent microangiographic radiographic studies as well as light histologic studies.

Mobilized and yet injured nerves kept electrical and histologic pace with those injured but not mobilized. Post injury conduction velocities and NAP amplitudes were comparable in both series. Patterns

of revascularization were identical except in the early weeks after injury where a larger quantity of both collateral and intraneural vessels were seen in the non-mobilized nerves than in those mobilized. Mobilization of acutely injured nerves should be minimal, but from these studies appeared safe since functional regeneration does not depend on the initial preservation of the collateral blood supply.

Muscle Power Studies

Although useful for measuring early regeneration, nerve action potential recording and, for that matter, end input recording of muscle action potentials, insertion potentials, deinnervational changes such as fibrillations and deinnervation potentials, and even nascent muscle action potentials do not indicate the degree of late functional reinnervation. For this reason, it was decided to supplement our EMG studies of end input reinnervation by a hopefully more objective measure of function, muscle power. A system was developed for the usual experimental nerve used in our studies, the tibial nerve, and for the gastrocnemius-soleus musculature. Initital studies were attempting by placing a tibial pin through the animal's leg and then securing the pin and leg within a cast. The foot was fixed to a pedal and a wire ran from the pedal to a strain gauge mounted on a brass bar. Activity of planter flexion registered by the strain gauge was modulated by a wheatstone bridge and then displayed on an oscilloscope. Nerve was stimulated to produce a supramaximal single twitch and than a tetanic response was measured. Results using this apparatus were not reproducible and as a result Doctor Bratton designed and built a more reliable system incorporating some of the ideas of this earlier device. His stainless steel and plexiglass apparatus included a hinged footplate which was connected by heavy stainless steel wire of constant length to a strain gauge mounted on a fixed but slightly flexible brass bar. The tibial pin was clamped to vertical bars which in turn were adjustable and could be fixed to longitudinal runners. Muscle contraction was measured by a modified isometric technique since the length of the muscle was relatively fixed with the foot dorsiflexed and held under some tension by the wire between the footplate and the brass bar. Resting tension remained relatively constant for each extremity as long as the baseline settings for positioning the limb in the strain gauge apparatus were duplicated. Deformation of the strain gauge itself was converted into electrical signals which were modulated by a wheatstone bridge fitted with fine as well as gross adjustment rheostats. Care was taken to zero the baseline before each muscle contraction recording. After establishing stimulus threshold, supramaximal stimuli were spaced from 1.5 to 2.0 per second. A series of single muscles twitch responses were received by a 3A9 differential amplifier and displayed on the oscilloscope as spikes and photographed with a Polaroid camera. Supramaximal or tetanic contraction was obtained by using stimuli at .08 to 0.12 milliseconds in duration at 200 to 300 stimuli per second. Nerve and muscle action potential baselines as well as those of muscle power were determined in both limbs of 32 primates, and at an interval of 1 hour to 52 weeks after injury. Limbs with mobilized non-injured nerves sustained small but definite decreases in muscle contraction strength, particulary if interval between operations was brief. Immediately after partial laceration, there was an averaged decrease in single twitch as well as supramaximal strength of 32.8% and 30.4% respectively while NAP velocity was maintained. One week following injury, muscle contraction decreased by a further 21.6% and 19.7% respectively while NAP velocity was decreased by 18.5%. Muscle power as well as NAP amplitude measurements were also reduced further than had been recorded immediately after injury by 2, 4, and 8, and to a lesser degree at 12 weeks. By 24 and 36 weeks post injury muscle contraction was less than pre-injury but not less than values recorded 15 minutes after injury. Values recorded at 52 weeks reflected a partial reinnervation of achilles musculature. Further studies over a 24-hour period following partial laceration demonstrated a significant pro-

gressive decrease in muscle power up to eight hours post partial laceration.

Neural activity following laceration appeared to fluctuate as function diminished during the early hours to weeks following injury. Histologic studies of proximal stimulus, laceration, and distal recording sites in both control and partially injured nerves was carried out by light and electron microscopic techniques. Each partially lacerated nerve had three zones of injury. The first, the zone of laceration, showed a complete neurotmetic change whereas the second which was adjacent to the laceration showed partial degenerative changes as in the earlier studies. The third zone which was most peripheral to the laceration was felt to have changes in ground substances with increased proteinaceous fluid and relative separation of the axons. The initial reduction in electrical activity seen with partial laceration is related, of course, to destruction of nerve fibers in the immediate region of the laceration. Further electrical deterioration is probably due to two factors: 1) continuing degeneration of nerve fibers in the intermediate zone adjacent to the laceration, and 2) a decrease in function of fibers in the peripheral zone which, although the fibers appear normal, may be in a abnormal environment with an increae in ground substance suggesting a proteinaceous edema fluid. Reconstitution of a blood nerve barrier by reconstruction of the perineurium leads to restoration of a stable endoneurial environment and probably causes the subsequent return of electrophysiologic activity towards that recorded immediately after laceration. The undamaged peripheral fibers and those undamaged fibers in the intermediate zone tend to resume their normal function. The laterimprovement in electrical function reflects regeneration of a portion of the cross of the nerve and this occurs predominantly in the intermediate zone where the damage was non-neurotmetic and scarring minimal. Regeneration also occurs but to a much lesser extent in the zone of the laceration where injury is neurotmetic and sbusequent scarring great. However, due to the neurotmesis found in this area and lack of regeneration because of it, full recovery does not occur even by a year.

Primary versus Secondary Repair for Transecting Injuries

Controversy has always existed concerning proper timing for transected nerves. After baseline NAP, MAP and muscle power studies were made, both tibial nerves were transected at a measurable distance from the ischial tuberosity. Nerve on one side was repaired primarily after sharply trimming each stump of a millimeter of tissue. Soft tissue wounds on both sides were closed and then the transected nerve of the opposite limb was repaired secondarily three weeks later. Proximal neuroma and distal glioma were sharply trimmed back to good fasicular tissue or, when stumps had grown together as they did in four cases, the resulting neuroma in continuity was resected. After mobilizing both stumps, the nerve was repaired with 6-0 nylon. The limb of each animal was re-evaluated electrically at one interval of 12, 16, 20, 24, 40, and 52 weeks and the neural specimens were studied histologically with Masson, Bodian, and Luxol Fast Blue stains. Although significant muscle function had not returned by 12 weeks post suture, NAP conduction velocity and amplitude favored primary repair in two of three animals. At 16 weeks, some reinnervation of muscle had occurred. Muscle power as well as NAP results were superior in limbs with primary repair (two of two). Tetanic contraction studies favored primary repair by an average of 0.85 kilograms. Two of three animals studied at 20 weeks had relatively advanced recovery on the primary side. The third animal had superior NAP conduction velocity on the primary but slightly better muscle power on the secondary side. Tetanic contraction studies favored primary repair by an average of 0.88 kilograms in the 24-week category although values were comparable

for both primary and secondary repair in one animal. Regenerative profiles were similar in two of three animals studied at 28 weeks while all studies favored primary repair in another. At 40 weeks, tetanic contraction differences between primary and secondary repairs were 1.4 and 2.9 kilograms respectively in two animals while in the third, conduction velocity and single twitch values favored primary repair even though tetanic contraction values were comparable. Data favoring primary repair held even at one year post repair. These studies supported work by Grabb and the suggestion by Seddon that nerves transected by glass can be repaired primarily although, of course, this data should not be construed as supporting primary repair for all nerve wounds such as those due to combat or blunt injuries to civilians for significant contusion favors secondary repair. Failure of secondary repair, at least in four instances, was due to interstump retraction without loss of external continuity at the repair site. Lack of proper fasicular matching due to resection prior to secondary repair may have been responsible for some of the relative failures although regeneration in the secondary group was eventually successful but not as superior as that in the primary group.

Blunt Injury to Nerve

A similar group of experiements was then carried out with blunt injury to nerve. Nerves were crushed by Ochsner forceps to the point where the evoked NAP's across the injury were flat. The crush injury on one side of the animal was resected and a primary repair was carried out whereas delayed resection and repair of the crush area on the opposite side was not. Fifteen animals were studied at an interval of 12 to 52 weeks post repair. Two of the secondary repairs distracted but in those animals without distraction primary was ahead of secondary repair in 10 of the 15 instances.

Repair Series

In 1972, Millesi reported on the use of interfasicular nerve grafts for repair of peripheral nerve injuries. He felt that tension on a suture repair led to scar and a poor result, and that "nerve regeneration after grafting without tension is much better than after direct end-to-end suture under moderate tension even though regenerating axons must cross two suture lines when grafts are used." Most surgeons have reserved the use of grafts for large gaps that cannot be repaired by mobilization of the nerve, sacrifice of unimportant branches, mild stretch (less than 10 to 15% of the mobilized length of the nerve), and proper positioning of the extremity. However, Millesi , Samii, Smith, and others have felt that results using autogenous grafts are better than those with end-to-end repair in almost all circumstances. Certainly, the superiority of autogenous nerve as a graft material has gradually been established over the years, but the question remains whether grafts should be used for all suture repairs. As a result, studies were initiated in this laboratory to compare interfasicular grafts in primates both with and without a gap in their tibial nerve with end-to-end interfasicular and end-to-end epineurial repair. Thirty adult Rhesus monkeys had both tibial nerves transected by broken bottle glass at the mid-thigh level and repaired using several techniques.

Repair series I- Interfasicular grafts versus interfasicular non-graft repairs(mild tension)

(These studies were in process when grant was terminated and have since been finished using University and Departmental funds for support.)

In 16 animals, interfasicular or fasicle-to-fasicle non-graft repair was done in one limb and interposition of one centimeter long interfasicular sural nerve grafts was done in the opposite limb. Baseline electrophysiologic studies included evoked nerve and muscle action potentials, and muscle strength measurements of planter flexion in response to single, repetitive and tetanic stimuli of the tibial nerve. Operative microscope was used for all nerve repairs. Suture used was 10-0 nylon for both the interfasicular grafts and for the interfasicular or fasicle-to-fasicle nerve repair. Each of the 16 animals was re-studied at one interval of 3, 6, 9, or 12 months post repair. Electrophysiologic recordings were repeated, nerves were fixed with glutaraldehyde in vivo and then removed for light and electron microscopic histologic examinations.

Upon re-exploration, nerve action potentials could be recorded from all nerves while planter flexion and response to tibial nerve stimulation could be recorded in each limb except one repaired by grafts and re-studied at only four months post repair. In four of the five animals re-explored at 4 months, muscle strength measurements were significantly better in the limbs with non-graft repaired nerves. On the other hand, values in the fifth animal were comparable in both limbs. In the three animals re-studied at 6 months, return of planter flexion was significantly better in each non-graft repaired limb. Differences between graft and non-graft repaired nerves at 9 months were not as significant as at 4 and 6. At 12 months, graft repair fared better in one, non-graft repair better in another, while in two animals, results were comparable.

When values are averaged at each time interval, limbs with the non-graft interfasicular or fasicle-to-fasicle repair were clearly ahead of those with graft repair at four and six months while there was no significant differences at nine and twelve months. Histologically, the grafted nerves showed a typical neuroma at the proximal suture line. When studing cross sections of the graft segment, there were well-defined segments with fasicular arrangement but a large number of regenerating axons were found in extrafasicular loci. Disorganization at the distal suture line was similar to that seen at the proximal one. Axons reaching distal stump fasicles were fewer in number and not as mature in the grafts studied at 4 to 9 months post repair as in the end-to-end repairs. The non-graft interfasicular repairs showed good fasicular pattern proximally, loss of fasicular pattern with neuroma formation at the suture line, and return of good fasicular pattern distally.

Since tension on the interfasicular non-graft repair was mild and presumably non-existant with the interposition of the grafts in the above experiment, it was felt that another experiment increasing the amount of tension on the repair site was necessary. As a result, in 13 animals, a one centimeter segment of tibial nerve was removed on either side. On one side, repair was done by end-to-end epineurial suture since fasicular dissection and repair was impossible due to the moderate degree of tension on the suture line. On the other side, a 1.2 centimeter length of sural nerve grafts were interposed as had been done in the prior experiment using an interfasicular technique. Baseline evoked nerve and muscle action potentials, and strain gauge studies of single twitch and tetanic muscle contraction were made prior to injury and repair, and then following injury and repair, again at one point in time in each animal at 4, 6, 9, and 12 months.

Tension was created on the side with end-to-end epineurial repair for three distractions occurred, one at four months, one at 6 months, and one at 12 months. In addition, there was one distraction

of a graft repaired nerve re-studied at 12 months. Muscle function studies showed a consistent pattern. At 4 months, 3 animals had a substantial difference in return of muscle function favoring the limbs repaired by the non-graft, epineurial method. At 6 months, epineurial repaired nerves had muscle contraction superior to those repaired by grafts. At 9 months, values were close with non-graft repaired nerve favored in one animal and graft repaired nerve in the other. At 12 months, only one of the three animals had continuity of both repairs, and in this animal the epineurial repair was favored. Values were averaged for each interval and epineurial repair was favored in 8 of the 13 animals, in 5 graft repair was favored, and in one there was no significant difference. Histologic regenerative patterns were as had been observed in the prior experiment. Despite increased tension on the non-graft or epineurial repaired nerves connective tissue proliferation was no greater than had been seen in the prior experiment. The addition of graft segments increased distance for such axons to travel by the length of the graft and this may account for the fact that regeneration in both of these sets of experiments seem to be ahead in the end-to-end repair group whether done fasicle-to-fasicle or epineurium-to-epineurium at 4 and 6 months. Later electrical values obtained at 9 to 12 months indicated that muscle function was increasinglu comparable indicating that regenerative rate in a repair using short grafts will eventually catch up. Nonetheless, it was equally clear that repair by grafts was not superior to that achieved by end-to-end repair unless distraction occurred.

The addition of a second suture line necessary for a graft repair presents a second opportunity for loss of axons which should be transmitted from the graft segments into the distal stump. In addition, it was apparent that even more proximally many axons escaped from the area on either side of the fasicle to interfasicular graft segment and took an "extra graft position". Nonetheless, axons did appear in the distal stump fasicles. The second suture line rather than presenting a second site for loss of axons may serve as a mechanism to recapture extrafasicular axons resulting from the first suture line of the graft segment.

It seems very clear from these primate experiments that use of short interfasicular grafts will work despite a delay in regeneration time compared to that obtained with end-to-end repair. Perhaps the role for such an interfasicular autologous graft technique is for repairs where the usual measures for gaining length have been used and a relatively short gap remains. Rather than stretching the stumps or placing the limb in extreme flexion, interposition of such grafts to make up short distances is feasible and regeneration, although somewhat delayed, will eventually succeed under these circumstances.

Fasicular versus Epineurial Repair

Equally important in the field of peripheral nerve repair is the hypothesis that fasicular repair promotes better regeneration and eventual function than does epineurial repair. In order to study this concept, 12 Rhesus monkeys had transection of the tibial nerves on both sides made after baseline evoked nerve and muscle action potentials, and single twitch and tetanic contraction muscle power studies. Transected nerve on one side was repaired with an epineurial end-to-end technique using 8-0 nylon whereas that on the opposite side was repaired using a fasicle-to-fasicle or intrafasicular repair using 8-0 nylon. Once again, animals were re-studied at one interval of 3, 6, 9, or 12 months. Function was comparable in three out of the four animals studied at three months, but muscle power could barely be recorded or not recorded at all in these animals. In one animal with better muscle

power return, epineurial repair was ahead. At six months, epineurial repair was ahead in each of the animals studied at that interval. Epineurial repair was ahead in one out of two animals studied at 9 months with the other animal being comparable and was clearly ahead in the two animals studied at 12 months.

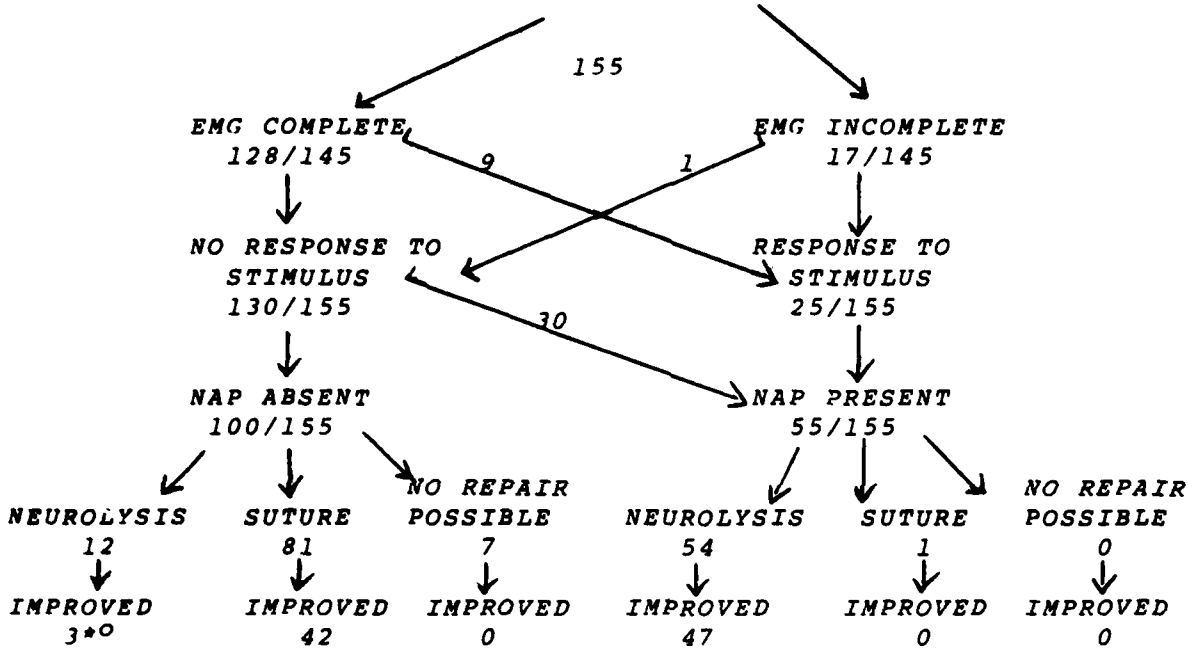
Suprisingly, the end-to-end epineurial repair seemed to produce a much more focal neuroma than was seen with a fasicular repair. With the latter, the neuroma seemed to begin where dissection of the fasicles began and seemed to end where that dissection ended so that grossly, fasicular repair produced a longer neuroma in continuity than did end-to-end epineurial repair. This occurred despite an attempt to preserve perineurium as much as possible around each fasicle. Histologically, both light and EM studies of cross section as well as occasional longitudinal sections suggested that axons were escaping fasicles both proximal and distal to the actual repair sites as well as at the repair site. This results in neuroma both above and below the actual end-to-end fasicular repair. This is, though, still a theory and awaits further proof by experiments to be proposed later in this request.

Conclusions gained, however, from these first three series of repair experiments included: 1) use of short interfasicular autogenous grafts for transected nerve offers no apparent advantage when compared with end-to-end non-graft repair done either by fasicular epineurial techniques providing distraction of the latter doesn't occur, 2) tension on a repair within the limitations of the experiments described does not appear to be an adverse factor providing once again distraction does not occur, 3) interfasicular or fasicle-to-fasicle repair does not offer an advantage over end-to-end repair although further experiments are necessary to see whether combining fasicle-to-fasicle repair with epineurial closure as well as experiments designed to study the fate of axons in a given fasicle and whether or not they escape that fasicle with fasicular repair are needed, 4) short interfasicular grafts do work and are capable of providing functional regeneration. Although classical techniques to make up length should still be utilized and end-to-end repair achieved, once these techniques have been expended use of short interfasicular grafts will hold some hope of functional recovery.

Much remains to be done including studies designed to provide further insight as to the reason(s) for relative failure of fasicular repair, effect of delay on repair, limits to graft length, tension studies, and biochemical features of regeneration through grafts.

as of June, 1975

TABLE I

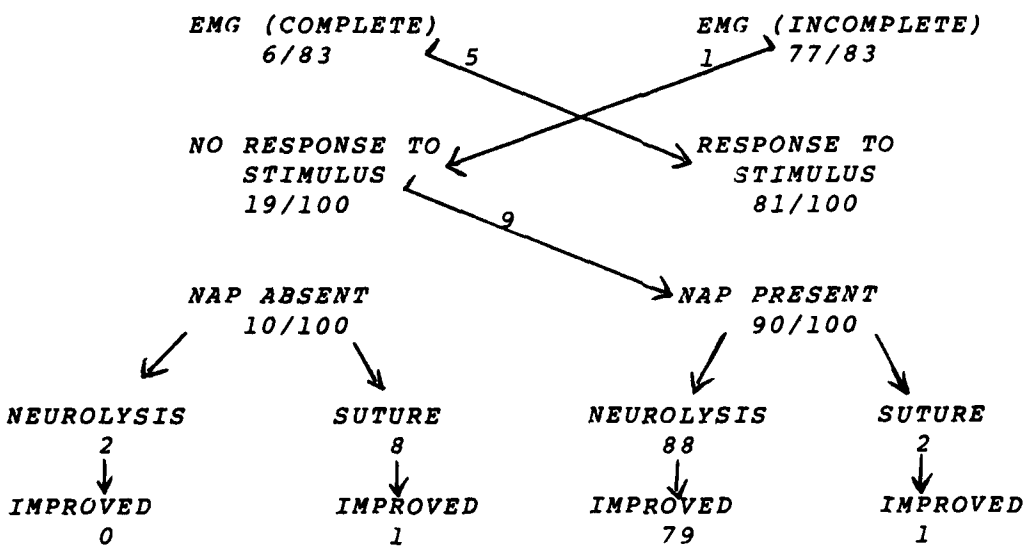


as of June, 1975

TABLE II

LESIONS FELT TO BE INCOMPLETE CLINICALLY

100



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Papers written as a result of Funded Research under contracts: DADA 17-69-C 9133 & DMD 17-76-C-6035

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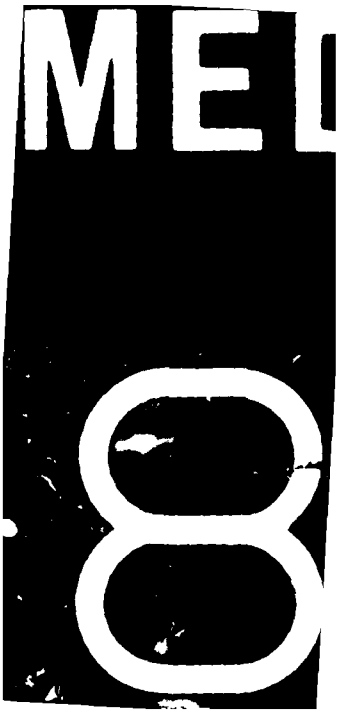
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